

## **CLAIMS**

This listing of claims will replace all prior versions and listings of claims in the application:

### **Listing of Claims:**

1. (CURRENTLY AMENDED) A porous silk fibroin material comprising a three-dimensional silk fibroin body having interconnected pores, wherein the mean pore size of the silk fibroin body is at least 225 microns ~~pores have a diameter of 10 to 1000 microns~~ and, wherein the material has a compressive modulus of at least 100 kPa.
2. (CANCELED)
3. (ORIGINAL) The porous silk fibroin material of claim 1, wherein the material has a compressive modulus of at least 150 kPa.
4. (ORIGINAL) The porous silk fibroin material of claim 1, wherein the material has a compressive modulus of at least 200 kPa.
5. (ORIGINAL) The porous silk fibroin material of claim 1, wherein the material has a compressive modulus of at least 250 kPa.
6. (ORIGINAL) The porous silk fibroin material of claim 1, wherein the material has a porosity above 80%.
7. (ORIGINAL) The porous silk fibroin material of claim 1, further comprising an additive.
8. (ORIGINAL) The porous silk fibroin material of claim 7, wherein the additive is a biologically active or pharmaceutically active compound.
9. (ORIGINAL) The porous silk fibroin material of claim 8, wherein the biologically active compound is a cell growth factor.
10. (ORIGINAL) The porous silk fibroin material of claim 9, wherein the cell growth factor is a cytokine.

11. (ORIGINAL) The porous silk fibroin material of claim 8, wherein the biologically active compound is a peptide that contains an integrin binding sequence.
12. (WITHDRAWN) A process for producing a porous silk fibroin material of claim 1, comprising the steps of:
  - a) forming a silk fibroin solution comprising silk fibroin in an aqueous salt solution;
  - b) removing the salt and water from the fibroin solution to form a silk fibroin substance;
  - c) forming a polymer solution comprising about 5 to 35% by weight of the silk fibroin substance in a solvent selected from a group consisting of hexa-flouro-iso-propanol (HFIP), N-methyl morpholine N-oxide and calcium nitrate-methanol;
  - d) contacting the polymer solution of step c) with water-soluble non-toxic particles that are insoluble in organic solvents and have a diameter between about 50 and about 1000 microns;
  - e) placing said polymer solution into a form;
  - f) removing the solvent from the polymer;
  - g) contacting said polymer solution with an effective amount of  $\beta$ -sheet structure inducing agent to induce  $\beta$ -sheet structure and insolubility in aqueous solution;
  - h) leaching said polymer with a solvent in which said particles are soluble and polymer is insoluble to remove said particles from said polymer; and
  - i) drying said polymer to form a porous silk fibroin material.
13. (WITHDRAWN) The process of claim 12, wherein step d) comprises placing said polymer solution of step c) into a form containing water-soluble non-toxic particles that are insoluble in organic solvents and have a diameter between about 10 and about 1000 microns.
14. (WITHDRAWN) The process of claim 12, wherein in step f) said solvent is removed by sublimation or evaporation.

15. (WITHDRAWN) The process of claim 12, wherein the silk fibroin is derived from a silkworm.
16. (WITHDRAWN) The process of claim 15, wherein said silk worm is *Bombyx mori*.
17. (WITHDRAWN) The process of claim 12, wherein the silk fibroin is derived from a spider.
18. (WITHDRAWN) The process of claim 12, wherein the silk fibroin is genetically engineered silk.
19. (WITHDRAWN) The process of claim 12, wherein the β-sheet structure inducing agent is selected from the group consisting of methanol, 2-propanol, 1-butanol, isoamyl alcohol, and chloroform, and acetone.
20. (WITHDRAWN) The process of claim 12, wherein the aqueous salt solution comprises lithium bromide, lithium thiocyanate or calcium nitrate.
21. (WITHDRAWN) The process in claim 12, wherein the particles are selected from the group consisting of alkali metal and alkaline earth metal halides, phosphates and sulfates, sugar crystals, water-soluble polymer microspheres and protein microspheres.
22. (WITHDRAWN) The process of claim 21, wherein the particles are sodium chloride crystals.
23. (WITHDRAWN) The process of claim 12, wherein the polymer is leached with water.
24. (WITHDRAWN) The process of claim 12, comprising the step of adding an additive.
25. (WITHDRAWN) The process of claim 24, wherein the additive is a biologically active or pharmaceutically active compound.
26. (WITHDRAWN) The process of claim 25, wherein the biologically active compound is a cell growth factor.
27. (WITHDRAWN) The process of claim 26, wherein the cell growth factor is a cytokine.

28. (WITHDRAWN) The process of claim 25, wherein the biologically active compound is a peptide that contains an integrin binding sequence.
29. (WITHDRAWN) A porous silk fibroin material produced by the method of claim 12.
30. (WITHDRAWN) A porous silk fibroin material produced by the method of claim 12 and a biocompatible polymer.
31. (WITHDRAWN) The material of claim 30, wherein the biocompatible polymer is selected from the group consisting of polyethylene oxide (PEO), polyethylene glycol (PEG), collagen, fibronectin, keratin, polyaspartic acid, polylysine, alginate, chitosan, chitin, hyaluronic acid, pectin, polycaprolactone, polylactic acid, polyglycolic acid, polyhydroxyalkanoates, dextrans, polyanhydrides, polymer, PLA-PGA, polyanhydride, polyorthoester, polycaprolactone, polyfumarate, collagen, chitosan, alginate, hyaluronic acid and other biocompatible polymers.
32. (WITHDRAWN) A method for producing a tissue engineered construct comprising culturing mammalian cells on a porous silk fibroin material produced by the method of claim 12.
33. (WITHDRAWN) A method for producing cartilaginous tissue comprising culturing multipotent cells on a porous silk fibroin material of claim 1 under conditions appropriate for inducing cartilage formation.
34. (WITHDRAWN) The method of claim 33, wherein said conditions comprise nonessential amino acids, ascorbic acid-2-phosphate, dexamethasone, insulin, and TGF- $\beta$ 1.
35. (WITHDRAWN) The method of claim 34, wherein said nonessential amino acids are present at a concentration of 0.1 mM, said ascorbic acid-2-phosphate is present at a concentration of 50 ug/ml, said dexamethasone is present at a concentration of 10nM, said insulin is present at a concentration of 5 ug/ml and said TGF- $\beta$ 1 is present at a concentration of 5 ng/ml.

36. (WITHDRAWN) A method for producing cartilaginous tissue comprising seeding multipotent mammalian cells on a porous silk fibroin material produced by the method of claim 1 and implanting said material into a host.
  37. (WITHDRAWN) A method for producing bone tissue comprising culturing multipotent cells on a porous silk fibroin material of claim 1 under conditions appropriate for inducing bone formation.
  38. (WITHDRAWN) The method of claim 37, wherein said conditions comprise ascorbic acid-2-phosphate, dexamethasone,  $\beta$ -glycerolphosphate and BMP-2.
  39. (WITHDRAWN) The method of claim 38, wherein said ascorbic acid-2-phosphate is present at a concentration of 50 ug/ml, said dexamethasone is present at a concentration of 10nM, said  $\beta$ -glycerolphosphate is present at a concentration of 7 mM and said BMP-2 is present at a concentration of 1 ug/ml.
40. – 59. (CANCELLED)